International Journal of Agricultural Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 7, Issue 1, Feb 2017, 327-334

ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF METHANOL EXTRACT OF BLEPHARIS GLOMERANS FLOWERS

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ABSTRACT

This study was undertaken to investigate in-vitro antioxidant and antibacterial activities of Blepharis glomerans flowers (BGF). In this experiment we used methanol for the soxhlet extraction. Methanol extract of BGF comprises alkaloids, cardiac glycosides, steroids, saponins, flavonoids and terpenoids. Phenol content of the BGF was 51.4 mg/g and flavonoid content was 38.9 mg/g. Methanol extracts of BGF showed DPPH (47.7%) and ABTS (44.8%) activity. Furthermore, the methanol extract was used in antibacterial studies, which shows the highest inhibitory activity against K. pneumoniae and least inhibitory activity against S. typhi. This study explored BGF as a potential source of antioxidant as well as antibacterial properties.

KEYWORDS: Blepharis Glomerans, Antioxidant Activity & Antibacterial activity

Received: Dec 07, 2016; **Accepted:** Jan 16, 2017; **Published:** Jan 28, 2017; **Paper Id.:** IJASRFEB201743

Original Article

INTRODUCTION

Vegetation reminiscent of herbs, shrubs and climbers are exploited for their bioactive compounds for human wellness (Ezzatzadeh et al., 2012). In addition, plants contain major bioactive constituents such as alkaloids, flavonoids, saponins, steroids, terpenoids, polysaccharides and tannins, which can be mostly contributing to biochemical events and latest therapeutic principles (Doughari et al., 2012). Herbal food supplements or alternative therapies or natural/plant based approach are being used to control various bacterial and fungal diseases (Prasannabalaji et al., 2012). The interaction of different groups of active metabolites within the extract could have enhanced the therapeutic outcomes more than the only components (Prasannabalaji et al., 2010). Oxidative stress is caused by an imbalance between the production of reactive oxygen and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage. Biochemical pathways or cellular mechanisms had been producing free radicals and reactive oxygen species as an end product (Nantitanon et al., 2007). Unstable chemicals are harmful to living cells, it can cause mutation, myocardial infarction, Alzheimer diseases and can be related to different scientific problems (Adiguzel et al., 2009; Sugumaran and Raj, 2010). Usually, chemical centered antioxidants are used to manipulate free radical activity and they have opposed results on human health and the development of normal antioxidant from plant assets could be fruitful to humankind (Rajasekar et al., 2011).

Blepharis the most important genus of Acanthaceae; contemporary monographs by way of Vollesen (2000) as containing 129 species. Quite a lot of species of Blepharis have confirmed medicinal values like anti-inflammatory, antiarthritic, antimicrobial, antifungal, antioxidant and cytotoxic properties. It was once pronounced that B.Edulis has been proven as a strong aphrodisiac undertaking, antispasmodic and antiplatelet aggregation (Fatima S et al., 2012). B.Ciliaris has shown antioxidant activity (Mohammed et al., 2012), and seeds have confirmed expectorant, aphrodisiac, and diuretic (Deshpande et al., 2006). Therefore the genus Blepharis has been validated for its medicinal values in previous studies.

MATERIALS AND METHODS

Plant Collection and Identification

Blepharis glomerans flowers were collected from the koyathanda at srisailam region, Andhrapradesh, India. The collected flowers were washed twice in running tap water to remove clay and grimes. Fresh flowers were lyophilized and then pulverized into powder form and sieved with 50 µm mesh. Methanol is used as a solvent in this experiment.

Hot Extraction Method by Soxhlet

20 g of plant powder was extracted in 500 ml of methanol (by soxhlet extraction technique overnight at 65°C temperatures). The acquired extracts have been subjected to vacuum evaporator to remove excess solvents. Then the dried crude extract yield has been weighed and used for additional experimental studies.

Phytochemical Screening of Methanolic Extracts

The small amount of crude methanol extract of *BGF* was dissolved in a suitable solvent and applied as a small spot on the activated thin layer chromatography (TLC) plate. The ensuing plate runs with solvent methods (100% chloroform, 70% chloroform + 30% n-hexane, 50% chloroform + 50% n-hexane, 30% chloroform + 30% n-hexane + 40% methanol) and visualized with quite a lot of spray reagents (vanillin-sulfuric acid spray, sulfate-sulfuric acid spray, Dragendorff's spray, aluminium chloride spray, 4-aminoantipyrine/potassium hexacyanferrate (III) spray, p-anisaldehyde–sulfuric acid spray, ethanolamine diphenylborate, chloranil reagent spray) to verify the presence of wide varieties of chemical materials such as alkaloids, glycosides, steroids, flavonoids, saponins, tannins and terpenes making use of standard methods (Touchstone JC et al., 1978; Jork H et al., 1994).

Determination of Total Phenolic Content (TPC)

Total phenolic content of *BGF* extract was determined by using Folin–phenol reagent method described by (Manian et al. 2008). A stock solution of plant extracts was prepared in different aliquots (0.2, 0.4, 0.6, 0.8, 1.0 mg/ml). Two ml of plant extracts was taken in test tubes and l ml of Folin–phenol reagent was added (FC reagent was dissolved in distilled water with 1:1 ratio). Then 5 ml of 20% sodium carbonate was added in each tube and subsequently the combination was blended competently with vortex mixer after which the experiment tubes were kept in the dark for 40 min. Absorbance spectra were recorded at 725 nm using glass cuvettes. To decrease commonplace error, the reaction was carried out in triplicate and the results were expressed in milligrams of gallic acid equivalent (mg GAE).

Determination of Total Flavonoid Content (TFC) by Colorimetric Process

Complete flavonoid content material of flower extracts was determined by Manian et al. (2008). Concisely, 100 µl of each and every plant extract (1 mg/ml) was once dissolved in corresponding solvents after which extracts had been made up to 1 ml using distilled water followed by the addition of 75µl of 10% sodium nitrate solution. After 6 min interval, 150

µl of 5% aluminium chloride solution and 0.5 ml of 1 M NaOH in test tubes was added. The mixture samples have been made as much as 2.5 ml by using distilled water. The spectrum absorbance values had been read at 510 nm. Results had been expressed as mg/g butylated hydroxytoluene (BHT) equivalents.

Antioxidant Evaluation

DPPH Assay

Free radical scavenging activity was determined by using the stable radical DPPH making use of the process of Siddhuraju and Becker (2003). Crude extract samples were set at 100 µg/ml, the plant extract of 2 ml was taken in test tubes and 3ml of 0.3 mM methanolic solution of DPPH was added, mixed well and allowed to incubate at 30^0C for 20 min. The absorbance value of the sample was measured at 517 nm. The percentage of DPPH radical scavenging endeavor was calculated by using the following formula

$$\% \text{ DPPH radical scavenging activity} = [\frac{\text{Reference OD} - \text{Sample OD}}{\text{Reference OD}} \text{ x100}]$$

ABTS Assay

The 2,2-azino-bis-three-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay is used for the determination of whole antioxidant activity of a plant extract. Inactive form of ABTS+ free radical cation is transformed to the lively form by means of reacting 7 mM ABTS aqueous solution with 2.4 mM potassium persulphate in the dark for 12–16 h at room temperature. The solution was diluted with methanol (1:89 v/v) and equilibtated at 30^0 C. Afterwards, 1 ml of ABTS solution was added with 100 µl of plant extract, and after an initial mixing of 30 min the absorbance value was measured at 734 nm. The percent of scavenging ABTS activity was calculated by using the following formula (Manian et al., 2008).

$$\% \text{ ABTS radical cation activity} = [\frac{(\text{Reference OD} - \text{Sample OD})}{\text{Reference OD}} \times 100]$$

Agar Disc Diffusion Method using B. Glomerans Flower Extracts

The methanol extract of BGF extract was checked for its potential biomedical application of antibacterial activity using the agar disc diffusion method. Gram positive and gram negative human pathogenic bacteria were used for the experiment and cultures were purchased from MTCC. Bacterial cultures of Escherichia coli (MTCC 1560), Staphylococcus aureus (MTCC 9542), Bacillus subtilis (MTCC 3055), Salmonella typhimuriun (MTCC 3224), Klebsiella pneumoniae (MTCC 2403), Enterobacter aeruginosa (MTCC 7661), Pseudomonas fluorescens (MTCC 2268) were used. Authentic bacterial cultures have been sub cultured in nutrient broth. The MHA nutrient agar was prepared with distilled water. The medium was autoclaved at 121^0C and at a pressure of 15 lbs for 20 min. The sterile medium was poured into autoclaved Petri plates and allowed to dry for a couple of minutes. Then, the aesthetic bacteria had been swabbed on agar plates. The surface of the agar medium is placed on a ready standard disc (streptomycin 50 µg/ml) as control. The stock flower extract was prepared in 100 mg/ml. After that, the crude flower extracts were prepared at various concentrations of 50 µl, 100 µl, 150 µl, and 200 µl and discs were soaked in the extracts overnight. After that, the disc was positioned in bacterial swabbed agar plates and incubated for 24 hours at 37^0 C. Subsequently, an incubation zone of inhibition was examined with the naked eye. Results have been found and the zone of inhibition (ZOI) was measured by using zone reader. The experiments were repeated thrice for better reproducibility.

Minimal Inhibitory Concentration (MIC)

MIC was determined by way of the microdilution process of Hammer et al. (1999) with minor changes. MIC is outlined as the lowest attention of drug which controls microbial population growth. In this work, we prepared different concentrations of methanol plant extracts (100, 50, 25, 12.5 mg/ml) to find out the strong concentration for inhibition of bacterial growth. The MIC assay was done using 96 well plate, filled with 50 µl of nutrient agar broth and 30 µl of bacterial culture, after which for treatment 30 µl of plant extract was added. Plates were incubated at 37^0 C for 24 h. Incubated plates were read at 560 nm in a microplate reader and values are tabulated.

STATISTICAL ANALYSIS

By using SPSS 19.0 software all statistical analyses were performed. All the quantitative data were presented as mean ± standard deviation (SD). Differences among means were analyzed by one-way ANOVA test, followed by Tukey HSD and student test. Statistical significance was set at $p<0.05$.

RESULTS

Identification of Secondary Metabolites, Phenolics and Flavonoids in *B.* Glomerans *Flowers*

Crude extract shown positive results of alkaloids, cardiac glycosides, steroids, saponins, flavonoids, terpenoids and phenolic compounds. Furthermore, methanol extracts of BGF phenolic and flavonoid contents of 51.4 mg GAE/g and 38.9 mg/g respectively.

Free Radical Scavenging Activity on *B.* Glomerans Flowers

DPPH and ABTS radical scavenging activity of methanol extract was shown in the below table.

Table 1: Antioxidants Activity of *B.* glomerans Flower Extract

Antioxidant Assay	Standard	Methanol
DPPH assay[a,b]	73.14± 0.07*	47.70 ± 0.07
ABTS assay[a,b]	81.31 ± 0.05*	44.80 ± 0.04

*Ascorbic acid.[a] Mean value (n = 6) with significant difference at $P < 0.05$. [b] Percentage of inhibition due to extract concentration of 100 µg/ml.

Mehanolic extract of BGF shown activity (47.7%) compared with the galic acid standard. ABTS free radical scavenging activity was analyzed using BHT as standard. Mehanolic extract of B. glomerulans showed activity (44.8%) compared with standard.

Antibacterial Activity of B. glomerulans

Methanolic extract of BGF shown higher inhibitor activity against K.. pneumoniae fallowed by E.coli, B.subtilis, entero bacter aeroges, pseudomans fluorescens.The least activity was measured in S.typhi and S. aureus. MIC range was calculated to be 25.0 mg/ml as the lowest concentration of methanol flower extract against K. pneumoniae.

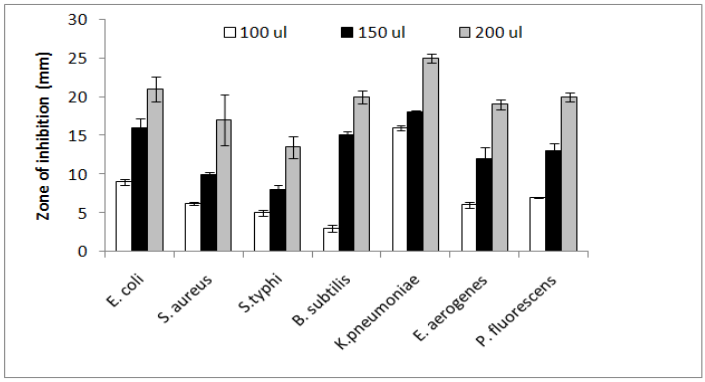

Figure 1: Antimicrobial Activity of Methanol Extract of B. Glomerulans

DISCUSSIONS

Several biochemical processes influence the overproduction of reactive oxygen species. Among all biochemical processes oxidative stress plays key role in the generation of free radicals. Elevated generation Of free radical formation brought on cell damage and prompted many dysfunctions like atherosclerosis, myocardial infarction, melanoma and neurogenerative disorders in human beings. But, common antioxidant compounds are helpful in repairing free radical formation in cells and properly manage various chronic disorders (Fakruddin et al., 2012). Antioxidant potential is based on the composition of exclusive phenolic contents present in the Scutellaria litwinowii extracts (Bazzaz et al., 2011). Antioxidant tests are incredibly targeted and touchy to temperature and incubation interval. Physiochemical properties of the sample are very crucial for analysing antioxidant potentiality.

Similarly, our results expressed methanol extract had moderate antioxidant activity observed using DPPH and ABTS methods in this concept, traditional antioxidants obtained a prominence as they're on the whole free from side effects, much less expensive and considerable in many plant sources (Cai Y and Luo Q, 2003). Tremendous quantity of medicinal plants were investigated for their antioxidant properties. Common antioxidants either within the type of raw extracts or their chemical components are very powerful to avoid the damaging tactics caused by using oxidative stress (Akinmoladun A.C et al., 2008; Özen T, et al., 2010). Recently, it has come to be evident that phenolic common products may diminish oxidative stress by way of oblique antioxidant action (Khatoon M et al., 2013; Islam S et al., 2003; Anjaneya S.R et al., 2012). Polyphenols were discovered in BGF. Plant polyphenols are synthesized from phenylalanine or from its precursor shikmic acid. These phenolics are major dietary antioxidants for the reason that they have got the ideal structural chemistry for free radical scavenging activities, and were proven to be extra powerful antioxidants in vitro than vitamins E and C on a molar basis (Ribeiro S.M.R et al., 2008). Polyphenols exhibit huge range of organic effects corresponding to safety of LDL oxidation in vivo with significant consequences in atherosclerosis and likewise guard DNA from oxidative damage with essential consequences in the age-associated progress of some cancers (Nidyaletchmy S.R et al., 2012). Our findings suggested that BGF rich in phenolic and flavonoid contents which are the major contributor to scavenge the free radicals in oxidation pathways.

The outcome acquired from correlation between polyphenols (phenol and flavonoid) and DPPH scavenging advised that phenolic compounds are the dominant contributors to the antioxidant properties of the extract/fractions. Additionally it is stated that secondary metabolites in the extracts corresponding to polyphenols, phenolic acids, flavonoids, diterpenes, tannins, phytosterols, fatty acid esters, phenylpropanoids, alkaloids and glycosides are major

bioactive compounds which have primary significance in medicinal chemistry (Maestri D.M et al., 2006). Hence, the antioxidant property may also come from different antioxidant presence in the fractioned extract as well.

Recently, the growing resistance rates of bacterial strains and manage of the progress of pathogens are huge challenge. Developing extra powerful antibacterial compounds using plant extracts is foremost in inhibiting bacterial growth rate. Clinically challenging S. Aureus strains are a most important reason of community and health related infections with an estimated mortality rate of around 7–10% (Tamokou et al, 2012). Methanol extract of BGF exhibited higher activity in K. pneumoniae, E.coli and B.subtilis. Plants contain bio flavonoids and phenolics, which are marker compounds for antimicrobial and antioxidant activities.

CONCLUSIONS

Crude methanol extract of B. glomerans showed antioxidant and antibacterial effect, it can be assumed that different active secondary metabolites were present in this extract, but the exact mechanisms still require further research.

CONFLICT OF INTEREST

We have declared that there is no conflict of interests in this study.

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